# Impacts of Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

**Michael CS Eacock** 

# **Public Comments**

No public comments were received for this proposal.

# **Technical Synthesis Panel Review**

# **Proposal Title**

#0241: Impacts of Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

Final Panel Rating	
inadequate	

# **Technical Synthesis Panel (Primary) Review**

### TSP Primary Reviewer's Evaluation Summary And Rating:

The authors make a strong case to better understand the role of deleterious effects anthropogenic contaminants on a federally-protected specie, the Giant Garter Snake (GGS). GGS populations in the Bay-Delta area have declined significantly, and the authors plan a study to assess the effects of two known contamints in the region. They propose to assess the effects of methyl Hg and Se on the GGS by using a surrogate specie, the Common Garter Snake (CGS) using an intensive laboratory study and plan to follow the lab work with an approach to ground-truth their results. They intend to use both biochemical and histiopathic approaches as indicators of Se and Hg exposure and effects. Their four-phased approach (diet study to evaluate effects of prey in field, reproductive success, possible antagonistic effects of Se on MeHg bioaccumulation, ground truthing by use of biomarkers) is innovative. There has been very little work done on the effects of MeHg on reptiles and the authors justify the need for such work. There were a number of major criticisms of the proposal. Two reviewers were quite concerned about the choice of the CGS for the surrogate species and associated problems with direct transferability of the results to the larger GGS. Bioaccumulation of metals in the GGS should be greater due to its ability to capture larger prey than the CGS, thus

### **Technical Synthesis Panel Review**

biomagnification would occur to a much greater degree in GGS than the surrogate. Second, reviewers were concerned about the lack of experience this group has with raising snakes in the laboratory, suggesting that it is a quite difficult task and the presentation of the plan did not express that confidence (one key vitae was missing from the proposal). While there is a paucity of studies dealing with the effects of mercury on snakes, there are many studies recently published on other reptiles that could have been woven into the study to better justify approaches to be followed. Finally, the ability to ground-truth the effects of Hg and Se are compounded by the fact that other contaminants in the region (pesticides, organochlorines) may make the biomarlers chosen non-specific to Hg and Se.

### **Additional Comments:**

The authors make a strong case to better understand the role of deleterious effects anthropogenic contaminants on a federally-protected specie, the Giant Garter Snake (GGS). GGS populations in the Bay-Delta area have declined significantly, and the authors plan a study to assess the effects of two known contamints in the region. They propose to assess the effects of methyl Hg and Se on the GGS by using a surrogate specie, the Common Garter Snake (CGS) using an intensive laboratory study and plan to follow the lab work with an approach to ground-truth their results. They intend to use both biochemical and histiopathic approaches as indicators of Se and Hg exposure and effects. Their four-phased approach (diet study to evaluate effects of prey in field, reproductive success, possible antagonistic effects of Se on MeHq bioaccumulation, ground truthing by use of biomarkers) is innovative. There has been very little work done on the effects of MeHg on reptiles and the authors justify the need for such work. There were a number of major criticisms of the proposal. Two reviewers were quite concerned about the choice of the CGS for the surrogate species and associated problems with direct transferability of the results to the larger GGS. Bioaccumulation of metals in the GGS should be greater due to its ability to capture larger prey than the CGS, thus

### **Technical Synthesis Panel Review**

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# **Technical Synthesis Panel (Discussion) Review**

### TSP Observations, Findings And Recommendations:

Impacts on Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

Researchers propose to look at mercury and selenium in a surrogate snake species, the common garter snake.

Reviewers questioned the approach of using the specific surrogate species. They would like to have seen a pilot study to validate the choice of the surrogate species.

Proposal was not hypothesis-driven. Resume of an important researcher was missing. Difference in bioaccumulation between the species could be expected. The species differences between the snake species in size and habitat concerns the reviewers.

The panel was both concerned about the choice of biomarkers (they are general biomarkers and offer little, specifically to MeHg and Se) and the choice of surrogate species. Researchers should better justify their choice.

Rating: inadequate

proposal title: Impacts of Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

# **Review Form**

### Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	This is a potentially important project. The authors have developed a strong circumstantial case for a role that various anthropogenic pollutants might play in adversely affecting populations of the federally threatened Giant Garter Snake (GGS). This premise is based on known affects of the listed contaminants on various other species of vertebrates that are intimately associated with aquatic ecosystems. However, not only have such effects not been examined for GGS, there are no baseline data regarding contamination levels for this or related species.
Rating	excellent

### **Justification**

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	The choice of Thamnophis sirtalis (the Common Garter
	Snake) is an appropriate surrogate for this project.
	Although T. sirtalis differs substantially from GGS in
	its ecology (principally, in being much less
	restricted to aquatic ecosystems and displaying
	broader diet preferences), these taxa are sufficiently
	related to justify their association in this

experimental context. Although, I might recommend to the authors that they consider Thamnophis atratus (Western Aquatic Garter Snake) from drainages due west of the Sacramento Valley as a better surrogate on the basis of closer phylogenetic and ecological relationships. However, T. sirtalis is attractive as an experimental subject given its ready commercial availability and relative ease of maintenance in captivity. As noted in their proposal, existing published research involving contaminant levels in non-CA populations of T. sirtalis suggests low sensitivity to metals; I would point out, however, that because different populations of T. sirtalis present different feeding ecologies (with respect to the degree to which they prey on aquatic versus terrestrial prey), they are likely to show considerable variation in levels of contaminants present in tissues. Based on what is known of the feeding ecology of GGS (feeding exclusively on aquatic prey species), a reasonable hypothesis is that GGS should show higher contaminant levels in bioassays.

Rating

excellent

# **Approach**

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments Although there is reference to T. sirtalis (the Common Garter Snake) as the surrogate species to be used in this study, in the description of Phase Two reference is made to an unrelated species, the Sierra Garter Snake (T. couchii).

On p. 9 (Task Six), reference is made to sampling Common and Aquatic garter snakes in the field. Authors should be clear which

species are being sampled. There are two species that co-occur with GGS over its range in the Sacramento Valley--the Common Garter Snake (T. sirtalis) and the Western Terrestrial Garter Snake (T. elegans). The Aquatic Garter Snake (T. atratus) occurs in creeks and streams (e.g., Cache and Putah Creeks) draining the eastern slopes of the northern Inner Coast Ranges but is not sympatric with GGS. Similarly, the Sierra Garter Snake (T. couchii) ranges to the lower foothills of the Sierra Nevada, but does not closely approach the range of GGS. There are likely populations of T. atratus that would be appropriate subjects for field sampling, especially those occurring at low elevations (base of foothills) and likely subjected to many of the same environmental contaminants as GGS.

P. 6 - It is unclear how much experience investigators have in captive husbandry of garter snakes. Although the experimental design appears sound, bear in mind that snakes are not the equivalent of lab mice and are much more challenging to raise, induce breeding, etc. Especially if the goal is to produce offspring, pre-gravid females will need to have a relatively higher food intake than males (this will affect number and size of neonates). Females will need access to a thermal gradient during pregnancy. Failure to provide "hot spots" for gravid females may result in stillborn or undersize offspring (as I learned with gravid GGS many years ago), thereby confounding data interpretation.

Rating very good

# **Feasibility**

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	See	last	comment	above.
Rating	very	good	i	

# **Monitoring**

If applicable, is monitoring appropriately designed (pre-post comparisons; treatment-control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	Controls are in place, and sample sizes seem adequate to provide a margin for "bumps" along the way (such as death of one or more lab snakes).
	very good

### **Products**

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	The potential development of a biomarker for field use is enormously important. This could serve as a means of monitoring the "health" of specific populations as well as identifying candidate areas for possible repatriation of GGS.
Rating	excellent

### **Additional Comments**

Comments	As clearly noted in the proposal, research involving
	contaminant effects in snakes is far from extensive.
	Particularly, the Giant Garter Snake, a Central Valley

endemic threatened with extinction, seems especially vulnerable to such effects. Populations have declined or disappeared from southern parts of the species' historical range in the central San Joaquin Valley in areas where aquatic ecosystems are relatively intact (e.g., Mendota Waterfowl Management Area, Fresno County). Such patterns of extirpation raise the prospect that environmental contaminants might play a significant role in these declines, a scenario that seems reasonable given the extraordinarily high levels of pesticide/herbicide applications present immediately surrounding GGS habitats.

# **Capabilities**

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	Project scientists have extensive experience with the contaminant end of the research. Unclear as to level of experience of personnel who will be involved with animal husbandry.
Rating	excellent

# **Budget**

Is the budget reasonable and adequate for the work proposed?

Commen	The budget appears reasonable and adequate for the research and products as outlined in the proposal.
Ratin	g good

### Overall

Provide a brief explanation of your summary rating.

nments
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There is strong justification for this research, based
not only on the relative endangerment of GGS, but also
because of the paucity of published data concerning
contaminant effects on aquatic reptiles. I would give
this proposal an "Excellent" rating if I could
ascertain a greater level of competence and knowledge
concerning the raising/breeding of snakes in the lab.

Rating very good

proposal title: Impacts of Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

# **Review Form**

### Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	The goals and hypotheses were clearly stated. The generation of data to understand the possible effects of Se and Hg on this endangered garter snake are timely and moderately important. I think the study has a good objective but was left wondering if all of the data was needed to assess the risk to the endangered garter snake.
Rating	very good

### **Justification**

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	1. The study seems justified. It would be useful to have more understanding of the effects of the "intense human settlement" on the garter snake populations. 2. The conceptual model was explained very clearly. 3. Honestly, the study addresses a good issue but I am left nervous about several issues. This suggests that the proposed work should be funded but a pilot studyor reduced study first would be best.
Rating	very good

# **Approach**

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	The proposed work will develop the tools and knowledge that it is intended to produce. There are no novel ideas created but, as discussed in the general ecotoxicology literature, information for reptiles is sparse. This work would add to a sparse body of knowledge. The information would be useful yo decision makers.
Rating	very good

# **Feasibility**

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

	The approach is definitely well documented and feasible. It is unfortunate that commerically-derived garter snakes that are different from the garter snake of concern must be used in the experiments. Could a more relevant garter snake species be used instaed?
	The authors will be able to complete the task as described and produce the information promised.
Rating	<del>-</del>

# **Monitoring**

If applicable, is monitoring appropriately designed (pre–post comparisons; treatment–control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	ΙĆ	do r	not	believe	that	this	issue	is	relevant
Rating	not	t ar	ppli	icable					

### **Products**

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	Inofmration for the endangered garter snake will be valuable. The application of this information to determining the contribution of Hg and Se to the decline in the garted snake population would be difficult given the unquanitfied impact of human development (habitat loss/modification.
Rating	good

### **Additional Comments**

Comments

# **Capabilities**

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	These authors clearly are capable of doing what they propose. I have no doubts or qualifying comments to make relative to these aspects of the proposal.
Rating	excellent

# **Budget**

Is the budget reasonable and adequate for the work proposed?

Comments	The	budget	does	seem	a	bit	high	for	the	${\tt information}$	to

	be generated.
Rating	very good

# **Overall**

Provide a brief explanation of your summary rating.

Comments	This is a very good proposal that identifies an important issue. The authors are very capable and there is no doubt that they would produce what they promise. The concerns that are expressed above suggest to me that funding for a pilot study would be best with an understanding that the group will apply for additional funding when the pilot study is completed and the information indicates a need for more information.
Rating	very good

proposal title: Impacts of Dietary Selenium and Methyl Mercury on Garter Snake Populations in the Sacramento and San Joaquin Watersheds

# **Review Form**

### Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	The goals and objectives of the experiment are clearly stated. Hypotheses while implied are not explicitly stated; but this does not detract, and is possibly not as relevant for this project due to the paucity of data in this system. The idea is timely, and the research has scientific merit based on the lack of data surrounding bioaccumulation/biomagnification in reptiles. Specifically, considering the high trophic level occupied by snakes, the project's goals are deserved of further investigation. However, the importance of the project while relevant to the examination of environmental influences on key species (of which T. gigas is one), might be moot considering the major threat to T. gigas populations is habitat loss.
Rating	excellent

### **Justification**

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	Due	to	the	paucity	of	data	a surround	ing	the	impact	s of
	sele	eniu	ım an	d methy	lme	cur	y on repti	les,	, spe	cifica	lly
	snak	ces;	the	study	can	be :	justified.	Hov	vever	this	same

lack of data could act as a hindrance to success, during some points of the project. See comments in "Approach" below. The conceptual model while not explicit, becomes apparent upon examination. The underlying basis for the proposed work is explained clearly. This project can be justified on the basis of lack of data, and the well established methodology being used to determine biomarkers (i.e. high likelihood of success).

Rating very good

### Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments The approach is well thought out, and will most likely prove successful. However, I question some of the inferences that must be made in this proposal. First of all, the use of T. sirtalis (as opposed to T. couchii)as a proxy for T. gigas is questionable. The life history traits of T. sirtalis are significantly different from T. gigas, and so this choice gave me reservations. Also, the time frame being examined while falling within the realm of a three year project might fail to yield the bioaccumulation levels necessary or similar to in situ levels and chronic exposure over a longer lifespan. Furthermore, being able to conclusively state that reproductive, teratological, or other impacts are due solely to exposure to inorganic toxics is notoriously difficult. Finally the small sample sizes in tasks 2.3, and 4will make statistical inferences difficult. As far as the approach involved (i.e. phases one through five) the project is well thought out. The results will most definitely add to the base of knowledge, and findings will be novel. Methodology and approaches are not as novel, but are proven and well established for this

	type of study, mitigating some of the previously
	mentioned sources of uncertainties. The development of
	the biomarkers in question will be completely novel,
	adding to the value of the project.
Rating	very good

# **Feasibility**

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	The approach is fully documented and technically feasible. The likelihood of success is very high. The scale of the project is consistent with the objectives and the authors have a very firm grasp of the issues at hand.
Rating	excellent

# **Monitoring**

If applicable, is monitoring appropriately designed (pre-post comparisons; treatment-control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

	Monitoring is inferred. In task 6 "ground-truthing" will be conducted in order to validate the biomarkers determined in task 5. Again the use of T. sirtalis as a proxy for T. gigas detracts from the project due to differences in life history. Development of the biomarker is valuable, and hopefully will prove successful. The development of this tool will aid in measuring the fate/transport
	tool will aid in measuring the fate/transport
	of selenium and mercury and their
	influence/adverse effects on key species.
Rating	very good

### **Products**

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	The stated deliverables from this project are three peer-reviewed manuscripts and three powerpoint presentations. Due to the importance of the findings and the ramifications into the fields of resource management and conservation biology, as well as the basic ecotoxilogical work being done, I would like to see more than this number of publications, especially when placed in the context of the desired budget. Interpretative /interpretable outcomes are very likely from this project.
Rating	very good

### **Additional Comments**

I have reservations regarding the use to captive born/raised T. sirtalis in place of the species in question (T. gigas). Due to the ESA classification of T. gigas, the choice is understandable, but I would have preferred to have the project carried out with a better proxy species (ie. field captured T. couchii as opposed to purchased T. sirtalis). Particularly when you consider biomagnification/bioaccumulation as a function of mass, and the disparity between T. sirtalis and T. gigas in this regard.

# **Capabilities**

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

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The proposal is supported by a large research group with ample experience and background to successfully complete this project. However, considering the large role that she will be playing, I was chagrined to see that Marti Wolfe was not able to get a CV in for the proposal submission. The team is extremely well supported/prepared to do the ecotox work. Also, they are accompanied by a seasoned management staff and a well prepared support staff. The infrastructure and support necessary to successfully accomplish the project are more than sufficient.

Rating excellent

# **Budget**

Is the budget reasonable and adequate for the work proposed?

Comments	Some of the budget requested within the individual tasks seems high. Specifically, the labor costs being requested by some participants are higher than anticipated (considering the stated positions of the researchers), while being quite low for others (i.e. research assistants). Budget requests for supplies,		
	reagents, and overhead are well within reason.		
Rating	very good		

### Overall

Provide a brief explanation of your summary rating.

Cor		This project has been well thought out, researched, and described. The development of biomarkers in T. sirtalis is a timely project, but one that has limitations due to the inability to work with the key species in question T. gigas. Furthermore, I question the logic in choosing the model species (T. gigas) as the primary threat to this species is habitat loss.
	the primary threat to this species is habitat loss.  However, it is a good choice from a trophic	

standpoint, and it will yield valuable data on the fate and transport of selenium and mercury, and how these inorganics impact key aquatic species. Therefore, the findings from this project while valuable, may be constrained to addressing issues more closely related to T. sirtalis rather than the intended species of interest, T. gigas. The team of researchers is well established and well qualified to complete this project. From a methodology standpoint, I see no reason why this project would not be successful. The team of researchers has the technical skill to complete the project, and the means to do so. When taking all factors into consideration, I would give the overall project a rating between very good and excellent.

Rating very good